RESEARCH NOTE

Detection of *Chlamydia trachomatis* DNA using MagNA Pure DNA extraction and Cobas Amplicor CT/NG amplification

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ABSTRACT

The automated MagNA Pure DNA extraction method for *Chlamydia trachomatis* was compared with the manual Cobas Amplicor protocol using 100 µL of input sample volume from 964 specimens. Agreement between protocols was 96.1%. The automated extraction method had a sensitivity of 99% and a specificity of 100%. Amplification inhibition observed after manual preparation of samples (3.8%) was not apparent following automated extraction. Using 200 µL of sample in the automated extraction process lowered the detection limit without raising the inhibition rate. Furthermore, the automated extraction method halved the hands-on time required for the procedure.

Keywords Automated DNA extraction, *Chlamydia trachomatis*, Cobas Amplicor, detection, inhibition, MagNA Pure

Original Submission: 10 January 2005; **Revised Submission:** 8 November 2005; **Accepted:** 11 November 2005

Clin Microbiol Infect 2006; 12: 576–579
10.1111/j.1469-0691.2006.01437.x

Lower genital tract chlamydiosis is the most frequent sexually transmitted bacterial infection among young people in the world [1]. Often asymptomatic, it can lead to inflammatory pelvic disease and severe reproductive disorders if untreated. The Cobas Amplicor system (Roche Diagnostics, Meylan, France) allows automation of the amplification and detection of *Chlamydia trachomatis* DNA, but the DNA preparation process remains manual. Using the MagNA Pure LC robot and DNA isolation kit I (Roche), a DNA preparation protocol (Table 1) was designed and compared to the Cobas Amplicor CT/NG manual extraction procedure.

During a 19-month period, 964 consecutive specimens (Table 2), 608 (63%) from women, were tested retrospectively using the two DNA preparation methods in parallel. Urogenital swabs were placed in 2-sucrose-phosphate (2SP) medium, while semen and first void urine (FVU) samples or peritoneal fluids (PF) were collected into sterile vials. All specimens were delivered to the laboratory within 4 h, where a 100-µL aliquot of PF was added to 2SP medium. All samples were stored at 4°C unless analysis was delayed for > 2 days, when samples were stored at − 80°C.

Aliquots of semen (100 µL), FVU (500 µL) or 2SP medium inoculated with a urogenital swab or PF (100 µL) were processed as shown in Table 1. In the second phase, manual extraction of 100 µL of inoculated 2SP and semen samples was compared with MagNa Pure extraction using aliquots of 100 µL and 200 µL. When discrepancies were observed between the PCR results obtained after manual extraction and MagNA Pure extraction, aliquots of the original sample stored at –80°C were re-extracted by both methods and re-amplified once.

Using 100 µL aliquots, the results with the two DNA preparation methods were concordant for 926 (96.1%) specimens (Table 2), of which 830 were uniformly negative. Thus the specificity of MagNA Pure extraction was 100%, taking manual extraction as the reference standard. No false-positives were detected following MagNA Pure extraction, in contrast with a previous study [2] that reported a variable proportion of false-positive results, depending on the type of sample analysed (0.1% of genital swabs; 1% of urine specimens). Among the 97 positive samples following manual extraction, 96 were positive following automated extraction. Thus, taking manual extraction as the reference standard, the sensitivity of MagNA Pure extraction was 99%.

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Table 1. Sample preparation protocols used for *Chlamydia trachomatis* PCR

Extraction method		Cobas Amplicor CT/NG manual extraction method				MagNA Pure automated extraction method			
Sample		Urogenital swab	PF (100 µL)	FVU	Semen	Urogenital swab	PF (100 µL)	FVU	Semen
Transport medium		2SP medium	2SP medium			2SP medium	2SP medium		
Volume processed		100 µL	100 µL	500 µL	100 µL	100 µL	100 µL	500 µL	100 µL
Pre-lysis steps	Washing step				200 µL[a]				
	Centrifugation[b]				10 min				
	Freezing step		– 80°C		– 80°C		– 80°C		– 80°C
	Dilution step			500 µL[c]	1000 µL[c]				
	Heating step (37°C)			15 min	15 min				
	Centrifugation[b]			10 min	10 min			10 min[d]	
Lysis steps	Lysis buffer	100 µL	100 µL	250 µL	250 µL			100 µL	
	Storage 20°C	15 min	15 min	15 min	15 min	Load 100 µL of specimen in a well of the sample cartridge			
	Centrifugation[b]			5 min[e]	5 min[e]	Run the DNA isolation kit I on the MagNA Pure LC robot			
	Heating step 90°C	10 min	10 min	10 min	10 min	Mix the 100-µL eluted extract with 100 µL of diluent[f]			

PF, peritoneal fluid; FVU, first-void urine.
[a]Phosphate buffered saline.
[b]× 12 500 g.
[c]Urine wash buffer.
[d]Save the pellet.
[e]Save the supernatant.
[f]Cobas Amplicor diluent buffer.

Table 2. PCR results for detection of *Chlamydia trachomatis* DNA in 964 urogenital samples according to the specimen type and sample preparation method

Specimen	No. of samples	No. of positive, negative and inhibited samples[a] according to the extraction method: Manual	MagNa Pure: Positive	MagNa Pure: Negative	MagNa Pure: Inhibited
Cervicovaginal swab	558	Positive	41	1	0
		Negative	0	508	0
		Inhibited	1	7	0
Peritoneal fluid	36	Positive	5	0	0
		Negative	0	31	0
		Inhibited	0	0	0
Urethral swab	94	Positive	12	0	0
		Negative	0	82	0
		Inhibited	0	0	0
Semen	224	Positive	0	0	0
		Negative	0	201	0
		Inhibited	0	23	0
First-void urine	52	Positive	38	0	0
		Negative	0	8	0
		Inhibited	0	6	0

[a]100-µL sample volumes processed.

One cervicovaginal swab was negative following automated extraction, perhaps because of the low number of DNA copies in the 100-µL aliquot, since this sample was positive after repeating the MagNA Pure extraction with a 200-µL aliquot of the same specimen.

To assess the detection limit of the two extraction methods, serial dilutions of pooled positive samples were extracted by the manual protocol (100-µL aliquots) and by the MagNA Pure protocol (100-µL and 200-µL aliquots). The highest dilution that was reproducibly positive was 1 : 8 with 200-µL samples, whereas a 1 : 4 dilution was the detection limit with 100-µL samples.

Amplification of 37 (3.8%) samples was inhibited following manual DNA preparation, whereas no inhibition was observed following MagNA Pure extraction, as has been described previously for DNA purification from other bacteria [3]. Among these 37 samples, one cervicovaginal swab proved to be positive, while the remaining 36 samples were PCR-negative. Semen and FVU were inhibited more frequently (10.0% and 11.5%, respectively). Variable inhibition rates (2.7% to 15%) have been reported previously for urine samples [4–9], especially female urine [5,10,11]. In the present study, among 52 FVU (38 male) samples, five of the six inhibited samples were from females, giving an inhibition rate of 37.5% for female samples, but only 2.6% for male samples. Mahony *et al*. [6] observed that PCR

inhibition with FVU samples was associated most frequently with β human chorionic gonadotrophin and crystals.

To investigate the effect on the inhibition rate of using a double volume (200 μL) of sample for automated DNA extraction, a complementary set of 90 consecutive specimens (30 cervicovaginal samples, 30 urethral samples, 30 semen samples) was amplified following manual extraction (100 μL) and automated extraction (200 μL). Six samples were positive and 77 samples were negative with both extraction methods. No sample contained inhibitors following MagNA Pure extraction of the 200-μL sample. Seven samples (three cervicovaginal samples and four semen samples) that contained inhibitors following manual extraction were found to be PCR-negative following MagNA Pure extraction. Therefore, because of the lower detection limit and lack of amplification inhibitors, the MagNA Pure method using 200-μL sample volumes was considered to be the most effective DNA extraction method.

Preparation of 24 urogenital swabs for *C. trachomatis* DNA detection took 45 min of hands-on time when using the MagNA Pure extraction protocol, compared with 1 h 30 min with the manual Cobas extraction protocol. The amount of time saved was even greater with other types of sample, such as FVU or semen, since automated extraction required only one manual pre-treatment step before processing, and therefore allowed easy preparation of all types of sample in the same run. Reagents and consumables costs were three-fold greater for the MagNA Pure extraction method (6.85 Euros/assay) than for manual extraction (2.05 Euros/assay), but technician costs were estimated to be two-fold less for MagNA Pure extraction (0.90 Euros/assay) than for manual extraction (1.81 Euros/assay). Absence of inhibitors following automated extraction avoided the cost of repeated assays, as well as delays in obtaining the corresponding results. With a 3.8% inhibition rate, an additional indirect cost of 0.45 Euros/assay must be taken into account when using the manual extraction method. Nevertheless, the overall cost of *C. trachomatis* DNA detection using automated extraction was 20% more expensive than the manual extraction method, including reagents for extraction and amplification, consumables and technician time. This should also be balanced against the fact that the MagNA Pure robot is capable of performing DNA extraction and PCR assembly without human manipulation, which should therefore help to prevent PCR contamination during routine work.

ACKNOWLEDGEMENTS

The authors would like to thank J. Debs and S. Niedergang for excellent technical assistance, Roche Diagnostics (France) for providing the Cobas Amplicor kits used in this study, and P. Rowe for English language revision of the manuscript.

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RESEARCH NOTE

Characterisation and molecular epidemiology of extended-spectrum β-lactamase-producing *Enterobacter cloacae* isolated from a district teaching hospital in Taiwan

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ABSTRACT

Enterobacter cloacae ($n = 110$) isolates from a district hospital in Taiwan were screened for extended-spectrum β-lactamases (ESBLs). In total, 17 ESBL-producers were identified, based on the combination-disk synergy test using cefotaxime and ceftazidime ± clavulanic acid. Investigation of ESBL genes in 33 ceftazidime-resistant isolates revealed the SHV-12 gene in the same 17 ESBL-producers. In addition, one isolate also carried the CTX-M-3 gene, and two isolates also carried the CTX-M-9 gene. No major epidemic clone of ESBL-producers was identified by pulsed-field gel electrophoresis. Routine screening for the ESBL phenotype, focusing on ceftazidime-resistant *E. cloacae*, should be undertaken in this area.

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Keywords CTX-M-3, CTX-M-9, *Enterobacter cloacae*, extended-spectrum β-lactamases, SHV-12

Original Submission: 3 June 2005; **Revised Submission:** 8 August 2005; **Accepted:** 7 October 2005

Clin Microbiol Infect 2006; 12: 579–582
10.1111/j.1469-0691.2006.01384.x

Enterobacter cloacae is a major nosocomial pathogen, with susceptibility rates to cefotaxime or ceftazidime in Taiwan of 35–60% [1,2]. In contrast, the fourth-generation cephalosporin cefepime has remained highly active against *E. cloacae* [2]. These resistance profiles suggest that AmpC derepression or hyper-production is conferring resistance to the third-generation cephalosporins [3]. However, production of extended-spectrum β-lactamases (ESBLs) by *E. cloacae* isolates in Taiwan [4,5] and worldwide [6–8] has been reported, raising the possibility of therapeutic failure with isolates apparently susceptible to cefepime [9]. Recently, SHV-12 carriage has been identified among *E. cloacae* isolates from two large (>1200 beds) hospitals in northern Taiwan [4,5]. Therefore, the aim of the present study was to identify and characterise any ESBLs present among the *E. cloacae* isolates from a district hospital (*c*.600 beds) in a rural area of central Taiwan.

During 2001, 110 non-repetitive isolates of *E. cloacae* were collected from specimens of wound pus (35%), urine (25%), sputum (20%), blood (10%), catheter tips (6%) and drained pus (4%). MICs for all isolates were determined by the agar microdilution method, with the ESBL phenotype being indicated by a reduction of ≥ 3 $\log_2$ dilutions in the MICs of either ceftazidime (CAZ) or cefotaxime (CTX) in the presence of clavulanic acid 4 mg/L [10]. Combination-disk synergy tests using CTX (30 μg) or CAZ (30 μg) ± clavulanic acid (CLA; 10 μg) were compared as a test for the presence of ESBLs, which was confirmed by a ≥5-mm increase in zone diameter size for CTX + CLA or CAZ + CLA vs. the zone diameter size when CTX or CAZ was tested alone [11]. The double-disk synergy test, using cefepime and amoxycillin-clavulanic acid disks (placed 20 mm